BIOLOGICAL BULLETIN

OF THE

Marine Biological Laboratory

WOODS HOLE, MASS.

Editorial Staff

E. G. CONKLIN—*Princeton University.*

GEORGE T. MOORE—*The Missouri Botanic Garden.*

T. H. MORGAN—*Columbia University.*

W. M. WHEELER—*Harvard University.*

E. B. WILSON—*Columbia University.*

Managing Editor

FRANK R. LILLIE—*The University of Chicago.*

VOLUME XLII.

WOODS HOLE, MASS.

JANUARY TO JUNE, 1922

Wahr
5-5-1923

PRESS OF
THE NEW ERA PRINTING COMPANY
LANCASTER, PA.

MAR 27 1922

BIOLOGICAL BULLETIN

OF THE

Marine Biological Laboratory

WOODS HOLE, MASS.

VOL. XLII JANUARY, 1922 No. 1

CONTENTS

		PAGE
GOWEN, JOHN W.	*Identical Twins in Cattle.*	1
BILLS, CHARLES E.	*Inhibition of Locomotion in Paramecium and Observations on Certain Structures and Internal Activities.*	7
JACOBS, M. H.	*The Effects of Carbon Dioxide on the Consistency of Protoplasm.*	14
HALL, F. G.	*The Vital Limit of Exsiccation of Certain Animals.*	31

PUBLISHED MONTHLY BY THE
MARINE BIOLOGICAL LABORATORY

PRINTED AND ISSUED BY
THE NEW ERA PRINTING COMPANY, INC.
LANCASTER, PA.

AGENT FOR GREAT BRITAIN
WILLIAM WESLEY & SON
28 Essex Street, Strand, London, W. C.

Single Numbers, 75 Cents. Per Volume (6 numbers), $3.00

Entered October 10, 1902, at Lancaster, Pa., as second class matter under Act of Congress of July 16, 1894.

Editorial Staff

E. G. Conklin—*Princeton University.*

George T. Moore—*The Missouri Botanic Garden.*

T. H. Morgan—*Columbia University.*

W. M. Wheeler—*Harvard University.*

E. B. Wilson—*Columbia University.*

Managing Editor

Frank R. Lillie—*The University of Chicago.*

All communications and manuscripts should be sent to the Managing Editor, the University of Chicago, Sept. 15th to June 15th, or Woods Hole, Mass., June 15th to Sept. 15th. Subscriptions and other matter should be addressed to the Biological Bulletin, 41 North Queen Street, Lancaster, Pa

Contents of Volume XLII.

No. 1. January, 1922

Gowen, John W. *Identical Twins in Cattle* 1
Bills, Charles E. *Inhibition of Locomotion in Paramecium and Observations on Certain Structures and Internal Activities* 7
Jacobs, M. H. *The Effects of Carbon Dioxide on the Consistency of Protoplasm* 14
Hall, F. G. *The Vital Limit of Exsiccation of Certain Animals* 31

No. 2. February, 1922

Bowen, Robert H. *Studies on Insect Spermatogenesis, III.* 53
Van Cleave, H. J. *A Determination of the Degree of Constancy in the Nuclei of Certain Organs in Hydatina senta.* 85
Van der Heyde, Han C. *Hemoglobin in Thyone briareus Lesueur* 95

No. 3. March, 1922

Allee, W. C. *The Invertebrate Course in the Marine Biological Laboratory* 99
Copeland, Manton. *Ciliary and Muscular Locomotion in the Gastropod Genus Polinices* 132
Uhlenhuth, E. *The Effect of Iodine and Iodothyrin on the Larvæ of Salamanders, III* 143

No. 4. April, 1922

Turner, C. H. *A Week with a Mining Eumenid: An Ecologico Behavior Study of the Nesting Habits of Odynerus dorsalis Fab* 153
Donisthorpe, Horace. *The Colony Founding of Acanthomyops (Dendrolasius) fuliginosus Latr* 173
Wheeler, W. M. *Observations on Gigantiops destructor Fabricius and Other Leaping Ants* 185

423715

AGERSBORG, H. P. KJERSCHOW. *The Relation of the Madreporite to the Physiological Anterior End in the Twenty-Rayed Starfish, Pycnopodia helianthoides (Stimpson)* ... 202

No. 5. MAY, 1922

CAMERON, A. T., AND O'DONOGHUE, C. H. *Retinal Reflexes of Narcotized Animals to Sudden Changes of Intensity of Illumination* 217
GRAVE, B. H. *An Analysis of the Spawning Habits and Spawning Stimuli of Chætopleura apiculata (Say)* 234
AGERSBORG, H. P. KJERSCHOW. *Notes on the Locomotion of the Nudibranchiate Mollusk Dendronotus giganteus O'Donoghue* 257
MORGAN, LILIAN V. *Non-Criss-Cross Inheritance in Drosophila melanogaster* 267

No. 6. JUNE, 1922

Twenty-Fourth Annual Report of the Marine Biological Laboratory 275
KOPEČ, STEFAN. *Studies on the Necessity of the Brain for the Inception of Insect Metamorphosis* 323

Vol. XLII. *January, 1922* *No. 1.*

BIOLOGICAL BULLETIN

IDENTICAL TWINS IN CATTLE?[1]

JOHN W. GOWEN.

The phenomenon of a sterile, female-appearing animal resulting as a co-twin to a normal male has called attention to the problem, Do identical twins occur in cattle?

Three lines of evidence are available in answer to this question. The first consists of anatomical evidence, the ovaries of a twin-producing cow during pregnancy. This is the most critical and most desired. The second consists in the study of the normal sex ratios occurring in multiple births of cattle in comparison with those occurring in species where identical twins are produced. The third method consists in a comparison of the color markings of twins of the same or opposite sex.

In his paper on the freemartin, F. R. Lillie[2] collects data on the condition of the ovaries of cows producing twins. He showed in 22 cases where both ovaries were obtained corpus luteum were present in each. In 11 cases where only one ovary was obtained 9 had corpus luteum in that ovary. The two pairs of twins whose mothers lacked corpora lutea in the remaining ovary were of the same sex, one pair being males, the other pair females. These last two pair of twins may be identical or monozygotic twins. This appears doubtful, for as the data of Cole presented later will show, the proportion of single ovaries ovulating twice when the other one does not ovulate should not be very far from the proportion indicated by Lillie's data, providing, of course, that one ovary may ovulate twice when the other does not ovulate at all.

Lillie furnishes other data on the sex ratio of intrauterine twins

[1] Papers from the Biological Laboratory of the Maine Agricultural Experiment Station No. 141.

[2] Lillie, Frank R., 1917, "The Freemartin; A Study of the Action of Sex Hormones in the Foetal Life of Cattle," in *Jour. Expt. Zoöl.*, Vol. 23, p. 371–422.

F. P.

beside that indicated above. The ratios which he reports for all his data are 19 male, 24 male and female, and 11 female, the methods adopted being such as to insure a random sample of the general population of cattle twins. Cole[1] has also furnished data on the sex ratios of cattle twins which he obtained from cattle breeders' records of 303 twins. The ratios of these cattle twins were 43 male, 165 male and female, and 88 females. Seven cases of triplets were also reported. Besides these statistics the Maine Agricultural Experiment Station has collected data on the frequency of the different sexes of twins as they occur in Maine.[2] These twin births were born to services for which this station had the service records. All services were traced in case the birth record did not appear at the expected time and either the proper birth record obtained, the cow found barren or the cow killed before parturition. These records should form a very homogeneous set of data in which the sex of the twins was governed only by chance. The data from these results show 5 males, 16 male and female, and 5 females. The University of Maine Animal Industry Department has always kept records of its herds since it was established. These records should be a homogeneous set of data wherein only chance was acting on the sex of the twin births as the service dates and the disposal of all animals are known. The ratio of the sex of these twin births in these data are 4 males, 4 male and female, and 3 females. These data summarized are shown in Table I.

TABLE I.

SEX RATIOS OF TWIN BIRTHS IN CATTLE.

Authority.	Males.	Male and Female.	Females.	*P*.	*P* for a 1 : 2 : 1 Ratio.
A. Cole	43	165	88	*AB*. 001	.045
B. Lillie	19	24	11	*BC*. 55	.29
C. This paper	9	20	8	*AC*. 25	.92

[1] Cole, L. J., 1916, "Twinning in Cattle with Special Reference to the Freemartin," Science, N. S., Vol. 43, p. 177.

[2] The coöperative data mentioned were collected by Dr. Raymond Pearl while at this Station. The records for the college herd were obtained from the Animal Husbandry Department by the author.

The comparison of the three frequency distributions is made under *P*. The data of Cole and Lillie clearly do not correspond, the male class in Cole's frequency distribution being proportionately small, whereas the male class in Lillie's data is proportionately large. The data of Lillie and this paper correspond rather well. Here, again, the chief trouble is in the proportional differences of the male classes. The data of Cole and this paper are not so very far different, the great discrepancy being in the male class. The data of this paper are clearly about half way between those of Cole and Lillie in the proportion of sex. Cole's data were obtained from breeders. It is well known that the breeders register only about one half as many males as females in their herd books. Without question Cole would not knowingly have taken data so loaded. It is conceivable, however, that the breeders from whom he obtained his data might have been influenced by this proportion.

If identical twins are lacking in cattle, the proportion of births should be 1:2:1, providing no disturbances like lethal factors exist in the stock. The comparison of the frequency distributions to this proportion are given in the second column of *P* for Table I. No excessive deviations from this proportion exist. The poorest approach to expectation is the distribution presented by Cole. Here, judging from the male and female twins and the female twins, the male twins should be more frequent. This evidence is opposed to that of expectation on the basis of the production of identical twins.[1] Thus in human twin births, where identical twins are produced, the proportions of sex are: males 234,497, male and female 264,098, females 219.312, according to Nichols.[2] This proportion corresponds to a ratio of 1.07:1.20:1. In species where identical twins are produced it is clear that the like sex should be produced more frequently than the unlike sex of twins, whereas in Cole's data the like sex, the males in particular, are in defect. The proportion of twins as given by Lillie's data is more nearly in accord with the expectation of some identical twins. However, it will be noted that the male class is in excess and the

[1] The explanation of Cole for this difference will be taken up later.

[2] Nichols, J. B., Memoirs of the American Anthropological Ass., I (1907), also in Newman, H. H., "The Biology of Twins," Univ. Chicago Science Series.

female class in defect of its expected ratio. The agreement between the proportions of twins for these data and the proportions of twins on the theory that they are distributed in accord with those of human twins, where identical twins are known to exist, shows a slightly poorer agreement ($P = .24$) than does the theory of 1:2:1. The data of this paper clearly show a distribution of 1:2:1, indicating that identical twins are seldom produced in cattle.

Independent confirmation of this hypothesis may be obtained. The American Jersey Cattle Club describes the animals registered. From the Herd Books of this Association 749 twin females, 168 twin males and 207 male and female were taken for a comparison of their color markings. These twins have been taken and their white markings, tongue color and switch color recorded. The by-laws of this Society in 1913 do not mention any requirement to indicate that the female twin can not be registered before she is proved a breeder. Besides these, 523 pairs of females, the offspring of the same sire and dam, have been tabulated for these colors. These last were found from the Registry of Merit tables in conjunction with the Herd Books. Table II. shows the distribution for these data.

TABLE II.

	Female Twins.		Male Twins.		Male & Female Twins.		Sister and Sister.	
	Absolute.	Percentage.	Absolute.	Percentage.	Absolute.	Percentage.	Absolute.	Percentage.
Alike in all particulars	449	59.95	103	61.31	117	56.52	300	57.36
One body mark different	22	2.94	8	4.76	5	2.42	19	3.63
Many body marks different	43	5.74	7	4.17	15	7.25	35	6.69
Tongue color different	36	4.81	12	7.14	15	7.25	33	6.31
Switch color different	20	2.67	7	4.17	8	3.86	12	2.29
Tongue and body color different	22	2.94	3	1.79	4	1.93	16	3.06
Switch and body color different	57	7.61	10	5.95	12	5.80	20	3.82
Switch and tongue color different	25	3.34	5	2.98	7	3.38	15	2.87
Different in all particulars	75	10.01	13	7.74	24	11.59	73	13.96
Total	747		168		207		523	

Table II. shows that the percentage of cows which are alike in

their body markings, tongue color and switch color are approximately the same for the four groups of cattle. The greatest difference is between the twins which are the males and the twins which are the male and female. Here the difference favors the like sex twins by about 5 per cent. It will be noted that the like sex twins resemble each other between 3.43 and 4.87 per hundred more than do the twins which are male and female. If the like sex twins are compared for resemblances with the sister, sister combination of column five, the difference becomes less. These facts all point to not more than a low percentage of identical twins in cattle if they exist at all.

If the differences are compared and their significance determined, it will be seen that none of the combinations, female twins, male twins, male and female twins or sister and sister, are significantly different. In fact, the differences are at no time greater than two times the probable error of such difference. If the whole distributions are compared by the χ^2 method, it is seen that they resemble each other closely. Such being the case, it can not be argued that twins of identical markings, tongue color or switch color exist in cattle.

In the Armadillo,[1] *Dasypus novemcinctus,* when the 4 offspring are known to be of monozygotic origin it has been shown that the degree of resemblance between the members of the quadruplet is very high. This resemblance is nearly twice as great as that which exists between brothers of known dizygotic origin. It is consequently fair to assume that the production of identical twins, in the sense that they arise from a single egg, will increase the proportion of identical offspring in like sex twins as compared with twins of unlike sex, or sister and sister combination. The above data have shown that the proportion of like sexed twins do not differ appreciably in the proportions which resemble each other from those which are of unlike sex or of sister, sister combination. Such being the case, it follows that identical twins (monozygotic) are rarely or never produced in cattle.

The importance of this conclusion as related to the freemartin problem has previously been discussed by F. R. Lillie[1] and need only be mentioned here. Hart, Cole and others have assumed that

[1] *Loc. cit.*

the freemartin is an undifferentiated male formed from the division of a single male-producing egg. Clearly, on this basis, these animals should be identical in appearance and the identical classes in the groups female twins, male twins and male and female twins should be large compared with those of the sister, sister class. As this is not so, it becomes doubtful if the hypothesis adopted by them will hold for the origin of the freemartin.

INHIBITION OF LOCOMOTION IN PARAMECIUM AND OBSERVATIONS ON CERTAIN STRUCTURES AND INTERNAL ACTIVITIES.[1]

CHARLES E. BILLS,

ZOÖLOGICAL LABORATORY OF THE JOHNS HOPKINS UNIVERSITY.

The study of *Paramecium* under magnifications sufficiently high to permit of observations on the activities within the living organism has been considerably hindered in the past by the excessive motility which this protozoön exhibits. A number of methods, physical and chemical, have been employed in attempting to overcome this difficulty; yet all of these methods are open to the objection that they are transient, or that they hinder the observation of certain activities, or even that they are severely limited in their application by the sensitiveness of the organism to protoplasmic poisons and osmotic changes.

Of the physical methods for quieting paramecia the following may be mentioned: (1) entanglement in zoöglea or other debris; (2) retardation of locomotion in gelatinous media; (3) inactivation at low temperature. The objections to these methods are patent: Entanglement affords an occasional arrest of activity; but in the infrequent cases where it is by chance efficient the animal is often obscured from view. Retardation in such media as quince seed jelly or gelatin is effective only when the gel is sufficiently thick to prevent the entrance of food particles at the peristome, in which case observation on this activity is impossible. Chilling may greatly reduce locomotion, but internal movements are at the same time unavoidably retarded.

The chemical narcotizing agents in general use for the lower animals are also in certain respects unsatisfactory for *Paramecium:* Methyl alcohol and chloral in concentrations sufficiently high to give any noticeable effect render the animal abnormal in appearance before paralysis sets in. Ethyl alcohol is open to the same objection, though to a less degree. Chloretone in concentrations

[1] This paper was prepared under the direction of Professor S. O. Mast.

around one tenth per cent. is serviceable; however, the margin between the narcotizing and lethal doses is so small that great precautions are necessary in its use.

In studying the action of several of the aliphatic alcohols the writer has observed that in propyl and iso propyl alcohols of certain dilution a balance of effects obtains such that the external (locomotor) cilia of *Paramecium* are inactivated before the internal activities of the animal are changed. Locomotion, therefore, can be reduced or stopped without appreciably affecting any of the visible activities which obtain within the animal itself.

In narcotization a single paramecium is added to a drop of the alcohol solution; or, better, one volume of a rich culture of mixed races is quickly and intimately combined with one volume of the alcohol of double the desired strength. For class demonstrations a number of cubic centimeters should be prepared by the latter method half an hour in advance and distributed to the students for study. The narcotized protozoa may then be observed under a cover glass or directly with a water immersion lens. When the animals come in contact with the stronger alcohol a few are immediately killed, while the majority at first exhibit avoiding reactions—they move erratically, become frequently bent in at the peristome, and may discharge a few trichocysts; then, as these reactions to the new stimulus cease, they become paralyzed and settle to the bottom. This final effect obtains in approximately half an hour from the application of the alcohol, the time varying with the race and condition of the individuals. The completely paralyzed paramecia exhibit little or no ciliary activity on the body, yet the cilia in the oral groove and at the peristome maintain a vigorous and coördinated movement; the undulating membrane of the œsophagus continues to direct the ingestion of food in approximately the normal manner; and the activities of the contractile vacuoles, food vacuoles and streaming protoplasm remain apparently normal for an extended period. With careful manipulation the narcosis can be continued for four or five hours, and these activities can be *gradually* retarded by heavy or prolonged narcotization. Thus it is evident that the study of *Paramecium* is greatly facilitated by the use of propyl alcohol as a narcotizing agent.

Paralysis is induced by the action of the alcohol on the cilia.

These may be inactivated, incoördinated or cytolyzed. If the narcotized animals are removed to a normal medium, those of them which are not too severely affected slowly recover. Recovery is indicated by a beginning of locomotion accompanying the reappearance of the body cilia. Whether these are actually regenerated or merely reëxtended and stiffened for swimming has not been determined.

TABLE I.

SHOWING THE OPTIMUM CONCENTRATIONS OF PROPYL ALCOHOL REQUIRED TO INHIBIT LOCOMOTION IN EIGHT PURE LINES OF *Paramecium*.

The percentages by volume are estimated from the conversion equivalents for ethyl alcohol.

Number of Culture.	Normal Propyl Alcohol.		Iso Propyl Alcohol.	
	Per Cent. by Weight.	Per Cent. by Volume.	Per Cent. by Weight.	Per Cent. by Volume.
P. caudatum 1	1.0	1.3	2.0	2.5
2	1.5	1.9	2.5	3.1
3	2.0	2.5	3.0	3.8
4	2.0	2.5	3.0	3.8
5	2.5	3.1	3.0	3.8
P. aurelia 1	2.0	2.5	3.5	4.4
2	2.5	3.1	3.0	3.8
3	2.5	3.1	3.5	4.4
Average	2.0	2.5	2.9+	3.7

It appears that the permissible variation from the optimum narcotizing concentration is greater with iso and normal propyl alcohols than with any of the quieting agents heretofore employed. With different cultures, however, the narcotizing concentration varies considerably, as the results presented in Table I. indicate. This table gives the optimum concentrations of the two propyl alcohols when used on various typical pure line cultures of *Paramecium caudatum* and *Paramecium aurelia*. It should be noted that the former species narcotizes more satisfactorily than the latter, and that wild cultures, or mixed cultures of pure lines, are more satisfactory than single pure lines of either species, since among the varied specimens of the mixed "populations" a considerable number which will narcotize properly can be much more readily found than in the more uniform specimens of the pure stocks.

By appropriate concentrations of propyl alcohol other microscopic animals can be narcotized, though with less advantage than *Paramecium*. *Paramecium bursaria* is paralyzed, but cyclosis also is stopped. The erratic movements of *Oxytricha* continue, though less frequently and energetically. *Spirostomum* gives acute avoiding reactions, twisting and bending so sharply at the peristome that the body is frequently severed. The cilia on the fragments continue to beat as long as any ectoplasm remains, but those on unbroken animals that are narcotized quickly disappear. *Stentor* becomes quiet, and some of the cilia at either extremity continue to beat; it contracts so much, however, that little can be seen within. *Colpidium* and *Colpoda* become quiet, but the internal activities also cease. The contractile flagellates—*Euglena*, *Peranema* and *Distigma*—are stimulated to pronounced euglenoid contortions; their flagella are frequently lost, and may be grown again in fresh media. In the case of *Peranema* the writer has observed the flagellum actually regenerate about one fifth its length in three hours. Of the rotifers, *Monostyla* narcotizes satisfactorily; many of the others, however, merely undergo contortions up to the point of death. The various larvæ and nematodes that were observed gave likewise results of no special interest. In general, then, it may be said that while propyl alcohol gives a more or less effective narcotization in a number of microörganisms, *Paramecium* is the only form studied in which internal activities of a strikingly obvious character obtained after narcotization.

Some paramecia, narcotized in accordance with the technique above indicated, were examined in detail. It was ascertained that narcotization greatly simplifies the study of familiar details—cyclosis, functioning of the vacuoles, structure and activities of the œsophagus, and feeding. Several new observations and corrections were made; these are indicated below with the topics to which they refer.

In a narcotized paramecium, lying with the oral groove up, and the anterior end pointing to "twelve o'clock," there can be seen a steady and vigorous cyclosis extending anteriorly along the right fold of the body (anterior stream) and posteriorly along the left fold (posterior stream). The writer noticed that the cyclosis may

at times accelerate at some part; this acceleration is local and limited and soon traverses the entire course, ceasing first where it began. The acceleration seems independent of the functioning of the contractile vacuoles, since it occurs at intervals much greater than those of vacuole closure. The contractile vacuoles, fixed to the ectosarc, can be seen to operate, as in normal animals, at intervals of from ten to twenty seconds.

The gullet of *Paramecium* is usually represented in the literature as a short and narrow tube tapering posteriorly to the region of the aggregation of food particles, at which position it expands into a sac of somewhat greater diameter than the gullet proper. This description is doubtless referable to the fact that in the dead animal the œsophagus presents an appearance quite different from that in the living. In specimens carefully killed, fixed and stained the gullet was found to be scarcely recognizable, although the other structural details seemed unimpaired. In animals freshly killed, but not otherwise treated, the gullet presents the appearance indicated in the literature. The writer has often observed that in paramecia being killed by dilute poisons a convulsive rearrangement in the protoplasm in the posterior half of the body chokes up the œsophagus and tends to elevate the oral depression. The gullet, therefore, shortens and constricts, and if a new food vacuole is aggregating at this time the distal end of the gullet naturally remains relatively larger than the shrunken duct itself.

In a narcotized living paramecium the gullet can be observed as a smoothly curved and posteriorly tapering tube, approximately one fourth the length of the body. It extends from the peristome posteriorly toward the aboral side, but not through the middle of the body, so that the distal end lies near the ectosarc; and as seen from the oral surface and the posterior end, it twists obliquely to the left of the longitudinal axis of the animal (Fig. 1, *a, b, c*).

The undulating membrane, or ciliated lining of the œsophagus, was superficially studied in a few animals which had stopped swimming, but still rotated feebly, revealing the gullet in all its aspects. In these animals the undulating membrane was still functioning rapidly, though apparently somewhat slower than in a normal paramecium. The undulatory motion can be likened to that of at-

tached ribbons blown in front of an electric fan, or to that of the succession of waves obtained by shaking and twisting a number of fixed ropes. In no case, however, even at the moment of the cessation of undulatory motion, was it possible to observe in the gullet the cilia themselves; only their wave-like motion could be

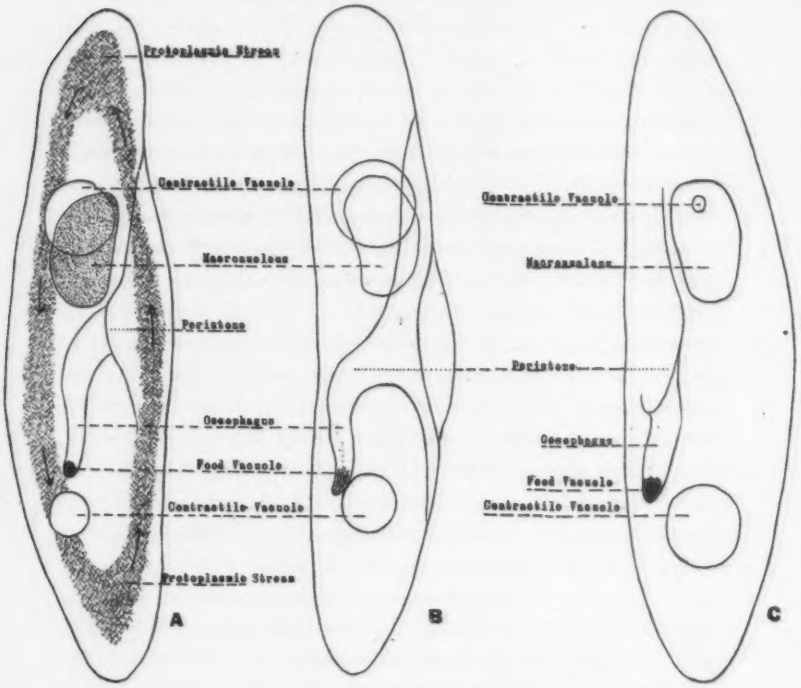


FIG. 1. Camera lucida tracings of specimens of *Paramecium caudatum* narcotized with propyl alcohol, intended to give especially the appearance of the gullet as the animal turns through 180°. The paramecia are heavily narcotized, and the incorporation of food vacuoles is beginning to be retarded.

A. View of paramecium in the position most frequently assumed when quiet. The gullet is in semi-profile. Food particles are aggregated, but remain in the gullet.

B. View of paramecium lying on its "side." The gullet is in full profile. Food particles are not aggregated.

C. View of paramecium from the aboral side—turned through 180° with reference to the position in (*A*). Food particles are aggregated, but remain in the gullet.

seen. The activities of the undulating membrane, in directing the passage of "food" particles (Chinese ink) to the vortex at the distal end of the gullet, and in returning unaccepted particles to the peristome, are rendered easy of observation by the quieting action of propyl alcohol. It may be, however, that the normal process of food acceptance is modified by the drug when sufficient of the latter is employed to effect a complete narcotization.

Summary.

1. Normal and iso propyl alcohols, in concentrations around two and three per cent., respectively, are superior narcotizing agents for *Paramecium*.

2. In narcotized paramecia it is possible to observe with convenience the structures and activities characteristic of the organism.

3. The cyclosis is not a uniform flow, but is subject to waves of acceleration.

4. The œsophagus is a much more extensive structure than is generally represented in the literature.

THE EFFECTS OF CARBON DIOXIDE ON THE CONSISTENCY OF PROTOPLASM.

M. H. JACOBS,

UNIVERSITY OF PENNSYLVANIA.

INTRODUCTION.

It has been shown by a number of recent investigators, using the microdissection and other methods, that the consistency of the protoplasm of different cells may vary from that of a liquid almost as mobile as water to that of a solid gel. It has also been shown that within the same cell at different times there may be almost equally striking differences. For example, Bayliss ('20) has reported that when the ectoplasm of an *Amœba* is observed with the ultramicroscope it appears to be filled with fine brilliant particles in rapid Brownian movement—an indication of the liquid state. On electrical stimulation the movement suddenly ceases, to be resumed when the stimulation is discontinued. Evidently in this case stimulation is attended by a solidification, or at least by a very considerable increase in the viscosity of the protoplasm. Another important example is furnished by cells in the act of division. Heilbrunn ('15) and Chambers ('17), using entirely different methods, have both shown that during the cleavage of marine ova there is with each cell-division a regular cycle of changes leading from a more liquid to a more solid condition and back again. It seems very likely that these changes play an important part in the actual mechanics of the division process. A third example is furnished by various cases of amœboid movement, where changes in protoplasmic consistency are thought by some of the most recent workers on the subject (especially L. Loeb, '02, '20, '21, etc.) to be concerned in actually producing the movement. A considerable number of other cases, some definitely established, others merely probable, could be mentioned. It seems certain that changes in protoplasmic consistency are not only of widespread occurrence, but are also of great physiological importance.

As to the cause of these changes, little is known. Heilbronn ('14) and Heilbrunn ('20) have shown that such effects can be produced by changes in temperature, but evidently this factor is not an important one in the cases that have been mentioned. Szücs ('13) showed that aluminium salts could bring about a solidification followed later by a liquefaction (both reversible) in plant cells, while Heilbronn, and especially Heilbrunn, have obtained striking results with ether, chloroform and other anæsthetics. Important as are these various studies on the effects of chemicals in throwing light on the nature of protoplasm, they give little information as to the cause of the physiological changes in viscosity observed under natural conditions, since the substances employed have not been such as are usually encountered in living cells.

In the course of some work done on a different problem several years ago the author observed certain effects of carbon dioxide on the protoplasm of living Protozoa which suggested the desirability of testing to what extent this substance might be able to cause reversible changes in protoplasmic viscosity. If it could be shown that it does, in fact, possess such powers in any striking degree, the observation would be a suggestive one, since CO_2 is not only a universal product of cell activity, but it has been definitely shown in certain cases (Lyon, '04) to be produced at different rates at different times within the same cell, and a plausible hypothesis might in that event connect the observed changes in viscosity, in part at least, with varying rates of CO_2 production. In the present paper evidence is presented that changes of the sort demanded by such an hypothesis may, as a matter of fact, be produced.

The material used consisted of the two Protozoa, *Paramœcium caudatum* and *Colpidium colpoda*, the eggs of the sea-urchin, *Arbacia*, and in a few experiments filaments of *Spirogyra*. In addition, a number of observations were made on *Amœba*. The consistency of the protoplasm of all of the cells except *Amœba* was tested by the centrifuge method of Szücs and of Heilbrunn, advantage being taken of the fact that the more viscous the protoplasm is, the more difficult it is to bring about a separation from it by means of centrifugal force of solid bodies of different specific gravity contained in it. The cells studied either contained bodies

capable of responding to this treatment (pigment and other granules and droplets of *Arbacia* eggs, chloroplasts of *Spirogyra*, certain fine granules in *Colpidium*) or such bodies could be introduced in the form of food vacuoles filled with India ink (*Paramœcium* and *Colpidium*). The force used with the *Arbacia* eggs was estimated at 1,600 times and with the other material at approximately 150 times that of gravity. The *Arbacia* eggs were centrifuged in sea water, *Spirogyra* in pond or in distilled water, and the Protozoa for the most part in their own culture fluid.

A portion of the experiments here described were carried out at the Marine Biological Laboratory at Woods Hole. The author wishes to acknowledge his indebtedness to the Director, Professor Frank R. Lillie, for placing at his disposal the facilities of the laboratory during a part of the summer of 1920.

Experiments on Paramœcium.

The procedure most frequently employed with this form was as follows: The animals were first placed in a test tube with about 20 c.c. of culture fluid to which a few drops of a dense suspension of India ink had been added. They were then allowed to stand for 30 minutes, at the end of which time they contained numerous black food vacuoles whose behavior could be followed with the greatest ease. Before exposure to carbon dioxide they were tested to determine the ease with which the food vacuoles could be separated from the remainder of the protoplasm. It was found in all the experiments with the culture used that a two minutes' exposure to a force of 150 times gravity caused a partial separation—*i.e.*, there was a general tendency for the ink to move toward the posterior end of the body—but it did not by any means become massed there, and in some individuals no movement at all was evident. When, at the end of 30 minutes, the animals had formed sufficient food vacuoles, a current of carbon dioxide was allowed to bubble through the liquid containing them, and samples were removed at intervals, immediately centrifuged for the same time and at the same rate as before, and then examined in Syracuse watch glasses. After this examination the watch glasses were in each case exposed to the air to allow the escape of the CO_2, and

after several hours another examination was made to determine whether or not deaths had occurred. A considerable number of experiments were made on *Paramœcium*, and as they all showed a very satisfactory agreement in their general results, it will be sufficient to describe in detail only the following typical one.

The animals used in this experiment responded to centrifugalization before treatment with CO_2 in the usual manner—*i.e.*, by a partial, but by no means complete, separation of the food vacuoles from the protoplasm when the force employed and the time of exposure to it were as already mentioned. After the current of CO_2 had flowed for one minute a slight increase in the readiness with which separation occurred was noted; this had decidedly increased by the end of 5 minutes, and by the end of 10 minutes it had become very striking. The India ink after centrifugalization was now invariably found in a compact mass in the posterior end of the body, so closely packed that the outlines of the individual food vacuoles could scarcely be distinguished. The animals themselves at this stage of the experiment showed no visible injury either from the exposure to the carbon dioxide or to the centrifugal force. When placed in open watch glasses they not only continued their locomotion in a normal fashion, but the food vacuoles within a few minutes had become redistributed throughout the cell and the animals had resumed their original appearance.

After 15 and 20 minutes, respectively, from the beginning of the experiment the results were essentially the same as those at the end of 10 minutes, except that a swelling of the body was now becoming increasingly apparent. At the end of 25 minutes a new appearance could be noted. In a few of the individuals, although the treatment was the same as before, the food vacuoles failed to separate. The numbers of such animals gradually increased during the next 15 minutes, until at the end of 40 minutes it was the exception rather than the rule for such separation to occur, and 5 minutes later only a few showed any movements of the food vacuoles at all. At 50 minutes this condition had become practically complete, and the protoplasm of the animals was now evidently considerably more viscous than at the beginning of the experiment. This increased viscosity—or perhaps solidification—

was also made apparent by the cessation of all movements of the contractile vacuoles. Swimming, however, continued, though at a slower rate than before.

All of the effects so far described were found to be completely reversible. Even after the protoplasm had passed through its period of liquefaction and had become much more solid than at the beginning of the experiment, the animals practically all recovered normally when the CO_2 was allowed to escape. From 56 minutes onward, however, evidences of fatal injury began to appear. The animals exposed for this length of time showed a mortality of about 25 per cent., though those that did not die recovered entirely normally. By the end of 65 minutes the mortality had increased to approximately 75 per cent., and 10 minutes later to 100 per cent. Since solidification began to be evident in some individuals in 25 minutes and was present in nearly all in 40 minutes, and since scarcely any deaths occurred before 50 minutes, it is evident that there is a considerable period within which the solidification is completely reversible. With an exposure of longer duration, however, it apparently merges imperceptibly into an irreversible death coagulation.

The effects so far described are much more rapidly produced if the animals have been kept in distilled water for a time before their exposure to carbon dioxide. For example, in one experiment a certain degree of liquefaction was produced in animals in the normal culture medium in 12 minutes; in individuals from the same culture which had previously been washed in distilled water this point was reached in less than 6 minutes. The times for the beginning of solidification in the two cases were 40 and 12 minutes, respectively, and while the animals in the culture medium were still living at the end of 60 minutes, those in the distilled water were dead in 32. These differences are perhaps partly due to the greater hydrogen-ion concentration of the distilled water, but experiments that need not be described here have shown that this is not the only factor concerned. The situation is, in fact, a complex one, presenting a number of points of interest, and its fuller discussion in another paper is contemplated.

At this point it may perhaps be well to consider two questions

that might justly be raised in connection with the facts that have been mentioned. The first is whether the behavior of *Paramœcium* may not be due to the removal of oxygen by the current of CO_2 rather than to the action of the latter substance itself; the second is whether the changes in the readiness of separation of the food vacuoles from the protoplasm may not be due to changes in the specific gravity of the latter brought about by the absorption or the giving off of water rather than to mere changes in viscosity.

As to the first question, it may be said that a vigorous current of hydrogen, which must remove the oxygen at least as effectively as the slower current of CO_2, was not found to bring about the sequence of changes described, even when allowed to flow for a considerably longer time. It did, to be sure, appear after a time to favor liquefaction of the protoplasm, but not only was its action in this respect far slower than that of carbon dioxide, but in no case were evidences of subsequent solidification observed though the experiments were continued much beyond the time required for the solidifying action of CO_2. As to the second of the two questions, it may be noted that there appears to be no evident correlation (as there should be if the chief factor were a change in the specific gravity of the protoplasm) between the degree of swelling in *Paramœcium*, which is evidently due to the taking up of water, and the ease with which the food vacuoles may be separated from the protoplasm. A second and even more convincing argument in favor of the view here adopted will be given when the experiments on *Colpidium* are described.

Experiments on Colpidium.

The behavior of *Colpidium* when exposed to carbon dioxide agrees in its essential features with that already described for *Paramœcium*. There is a preliminary period of liquefaction followed by increasing reversible solidification, and finally an irreversible coagulation. *Colpidium* is, however, more resistant to CO_2 than *Paramœcium* and the time required to bring about the final coagulation is considerably greater. This difference is especially striking when the animals have first been placed in distilled water—in fact, it is so great in that case that it may be put to

practical use in converting a mixed culture of *Paramœcium* and *Colpidium* into a pure culture of *Colpidium*. In case it is desired to make such a separation, it is only necessary to wash the animals in distilled water, concentrate them by gentle centrifugalization, and then introduce them suddenly into distilled water one half saturated with carbon dioxide. At the end of a few minutes *Paramœcium* will be found to be dead and *Colpidium* living; the CO_2 may then be removed from the water, a little dry hay added, and an excellent pure culture of the latter organism will be obtained.

Another minor difference between *Colpidium* and *Paramœcium* is that the former in its natural state appears to offer greater resistance to the separation of the food vacuoles by centrifugal force. While an exposure of two minutes, in the culture used, brought about a very distinct separation in *Paramœcium*, one of four minutes was only slightly effective in the case of *Colpidium*. One result of this greater difficulty in bringing about separation in the normal animals is that while it is very easy in *Colpidium* to demonstrate the preliminary liquefaction followed by an increasing solidification which finally leads to a viscosity at least as great as that at the start of the experiment, it is not possible to show conclusively, as in the case of *Paramœcium*, that this viscosity produced by CO_2 is actually greater than that in the untreated animals.

A third point of difference between the two forms is that in *Colpidium*—at least in the individuals studied—there are present, in addition to the food vacuoles, many fine dark particles scattered irregularly through the protoplasm. The effect of centrifugal force after liquefaction has occurred is to throw these particles to the anterior end of the body, while the food vacuoles containing ink go to the posterior end, giving a very distinct banded appearance to the animals, the anterior band being gray, the middle one transparent and the posterior one black. The appearance is very striking and can be seen even with a hand lens. With the centrifugal force used it was never obtained except in animals that had been exposed a moderate length of time to carbon dioxide. In individuals not exposed at all, or exposed a longer time, the banding was indistinct or entirely lacking.

This characteristic of *Colpidium* is of some importance in connection with the question already raised as to whether the differences in the ease of separation of the food vacuoles after exposure to CO_2 are due to changes in the viscosity or in the specific gravity of the protoplasm. In the case of the fine particles and the food vacuoles of *Colpidium* we are evidently dealing with two materials of different densities—one heavier and the other lighter than the protoplasm. If a change in the specific gravity of the latter through the taking up or giving off of water were alone concerned in facilitating separation, it is evident that a change that would favor the movement of the food vacuoles would hinder that of the fine particles and vice-versa. As a matter of fact, the movement of both is favored at the same time. The logical conclusion, therefore, is that a decrease in viscosity rather than a change in specific gravity is concerned, though it is by no means unlikely that changes in specific gravity—whose effects are, however, relatively insignificant—may also occur.

In concluding the section on *Colpidium* it may be worth while to present in tabular form the behavior of this animal and of *Paramœcium* in a typical experiment where both organisms were obtained from the same culture and were subjected together to the effects of CO_2.

Experiments on Arbacia Eggs.

Certain experiments, as yet unpublished, which the author has made with a different end in view on the eggs of the sea-urchin, *Arbacia,* confirm to a certain extent the conclusions arrived at in the preceding sections. The experiments were primarily intended to throw light on the relative efficiency of buffered solutions of the same pH, but containing different amounts of CO_2 in producing internal changes in cells. For the purpose of detecting such changes, advantage was taken of the readiness with which centrifugal force brings about a stratification of the materials found in *Arbacia* eggs into four layers. This appearance was first described by Lyon ('07) and has since been used with good effect by Heilbrunn in following the changes in the viscosity of the protoplasm of these eggs at different stages of division, under the effects of anæsthetics, etc.

TABLE I.

EFFECT OF APPLYING CENTRIFUGAL FORCE OF 150 TIMES GRAVITY AFTER VARIOUS EXPOSURES TO CARBON DIOXIDE.

Time of Exposure.	Paramœcium.	Colpidium.
1 minute.....	Slight evidences of liquefaction.	Slight evidences of liquefaction.
5 minutes.....	Liquefaction more distinct; body somewhat shrunken.	Liquefaction more distinct.
10 minutes.....	Practically all individuals show complete separation of food vacuoles.	Liquefaction very clear.
15 minutes.....	About the same; body beginning to swell.	Separation of food vacuoles almost complete.
20 minutes.....	Same; body more swollen.	Same; body somewhat swollen.
25 minutes.....	A few show failure of food vacuoles to separate.	Same as 20 minutes.
30 minutes.....	Same as 25 minutes.	Same as 20 minutes.
35 minutes.....	Same as 25 minutes.	A few show incomplete separation.
40 minutes.....	Many show no separation.	Less complete separation.
50 minutes.....	Most individuals show no separation.	More show absence of separation.
50 minutes.....	Absence of separation in practically all; recovery almost 100 per cent.	Separation absent in most individuals; recovery almost 100 per cent.
56 minutes.....	Same as 50 minutes; recovery about 75 per cent.	Same.
65 minutes.....	Same; recovery about 25 per cent.	Same.
75 minutes.....	All dead.	Same; many recoveries.

It is easy to demonstrate that carbon dioxide influences profoundly the consistency of the protoplasm of *Arbacia* eggs. In numerous experiments a five-minute exposure to sea water practically saturated with this gas was found to prevent stratification almost completely when the eggs were centrifuged for two minutes with a force estimated at 1,600 times that of gravity, while the controls in normal sea water showed the typical separation described by Lyon. That this result is due not to the hydrogen-ion concentration of the external medium as such, but rather to its carbon dioxide content (the carbonic acid probably penetrating the egg in an undissociated form or as CO_2 and subsequently dissociating within the cell), is shown by a comparison of the effects of

sea water to which CO_2 has been added with those of a similar solution in which the same pH has been produced by adding another acid—*e.g.*, HCl,—cautiously, drop by drop, and shaking vigorously after each addition to remove the CO_2 set free from the bicarbonates by the acid.

The results of one experiment of this sort may be given here. The exposures to carbon dioxide and to centrifugal force were those already mentioned. The numerical values of the pH of the solutions are only approximate, since the "salt errors" of the indicators used for determining them were disregarded, but as the error was in each case the same for the two solutions which were being compared, the general results obtained were not thereby affected.

TABLE II.

EFFECTS OF CENTRIFUGAL FORCE ON *Arbacia* EGGS EXPOSED FOR 5 MINUTES TO SEA WATER OF DIFFERENT pH VALUES.

pH (approximate)	pH regulated by adding CO_2.	pH regulated by adding HCl and then removing CO_2.
8.0	Stratification complete.	Stratification complete.
7.0	Stratification complete.	Stratification complete.
6.0	Stratification decidedly incomplete.	Stratification complete.
5.5	No stratification.	Stratification complete.

It appears from this experiment that the effects of carbon dioxide are primarily internal and depend on the absolute concentration of this substance rather than on the pH of the solution. These results are in agreement with those obtained by the author with other material and described in previous papers ('20-*a*, '20-*b*).

The solidifying effect of CO_2 is at least to a certain extent reversible. In several experiments eggs exposed to saturated sea water for 5 minutes were divided into two lots. The first lot was tested immediately and found to be solidified, giving no evidence of stratification after centrifugalization. The second lot was allowed to stand exposed to the air in a shallow dish for 30 minutes to permit the CO_2 to escape, or in some cases fresh sea water was also added. In either event centrifugal force produced normal stratification, showing that the eggs had again returned to the liquid

condition. Even the same eggs which had been centrifuged after exposure to CO_2 and had failed to stratify showed the normal behavior when the process was repeated after they had stood exposed to the air for 30 minutes.

It seemed desirable to determine whether in addition to this physical reversibility there was also complete physiological reversibility in the sense of permitting normal development to occur after solidification had been brought about. To test this point, a suspension of sperm was added to various lots of eggs after the CO_2 had been allowed to evaporate and the eggs had regained their fluidity. The results of these experiments were somewhat conflicting. In some cases no development occurred, in others there was cleavage as far as the 4- or the 8-cell stage, with perhaps irregular development beyond that point, while in still others normal development was obtained. The probable explanation of these differences was soon found to be that the exposure to CO_2 caused membrane formation (as had been noted by a number of previous observers) and subsequent development depended on whether or not conditions were favorable for complete artificial parthenogenesis. But whatever may have been the factors concerned in bringing about development, the mere fact that it occurred even in a portion of the cases where the eggs had undergone a very noticeable solidification is definite proof of the possibility of physiological reversibility.

It will be noticed that there have been described no liquefaction effects on *Arbacia* eggs. Whether or not such effects can be produced by lower concentrations or shorter exposures or both can be determined only when suitable material is again available. The experiments here described were made with another end in view before conditions in *Paramœcium* and *Colpidium* had been investigated, and the possibility of a preliminary liquefaction before solidification was not suspected. Though the experiments are, therefore, to this extent incomplete, their results, as far as they go, are in agreement with those obtained with the other kinds of material studied.

Experiments on Amœba.

The possibility of producing definite changes in the consistency of protoplasm by means of CO_2 suggested an interesting applica-

tion in the case of *Amœba*. It has repeatedly been urged by L. Loeb ('02), ('20), ('21), etc., that amœboid movement is due to changes in protoplasmic consistency. Hyman ('17) has also expressed the same view. The fact that carbon dioxide has been shown to cause such changes in the cases just described, and the additional consideration that it must be produced in an amœboid cell at a rate that varies greatly with circumstances, give some grounds for the hypothesis that the agent chiefly concerned in bringing about the protoplasmic changes underlying amœboid movement might be none other than this substance.

To determine to what extent the formation of pseudopodia can be influenced by carbon dioxide, an extremely fine capillary was made through which CO_2-saturated water could be discharged in small amounts. On several occasions quiescent amœbas (belonging to a species similar to, but probably not identical with, *A. Proteus*) were found, and on gently discharging a very small amount of the solution of CO_2 against the body of the animal pseudopodia were immediately put out in the direction of the capillary. On at least eight other occasions a small amount of the solution was discharged against the hindmost portion of a creeping amœba. In every case the same result was obtained. Pseudopodia were first put out at the point where the CO_2 came in contact with the body, the currents in the other pseudopodia being for a time reversed. Then as diffusion occurred and the full effects of the CO_2 became apparent all movements ceased for a few seconds, the animals remaining, as it were, congealed in the form in which they happened to be when overtaken by the effects of the dissolved gas. This paralysis then quickly passed away and movements were resumed in the original direction. To all appearances at least the animals had undergone a local liquefaction followed by a more general solidification. It follows from these rather crude experiments that if the external application of CO_2 can produce such results, it is not unlikely that internal effects of the same sort, resulting from varying rates of metabolism in different parts of the body, could conceivably give rise to amœboid movements of the usual type, though further work will be required to determine how far such an hypothesis explains the observed facts.

Experiments on Spirogyra.

It was thought desirable to supplement the experiments on animal cells with similar ones on some type of plant cell to determine how general the results already obtained might be. For this purpose *Spirogyra* was chosen, as it had already been successfully employed in a similar fashion by Szücs in his work with aluminum salts, and preliminary experiments showed that CO_2 produces marked differences in the ease with which the chloroplasts can be displaced by centrifugal force.

The first lot of *Spirogyra* studied, which was obtained early in the winter, showed clearly both liquefaction and solidification effects just as the Protozoa had done. Unfortunately, before all of the details of these processes could be studied, the material was exhausted and no more could be obtained until the following spring. This latter material, while giving the liquefaction effect with the greatest clearness, for some reason failed to show solidification except such as was obviously associated with irreversible death changes. The experiments, therefore, leave something to be desired in the way of completeness, but since the results, as far as they go, especially those obtained with the earlier material, are in good agreement with those already described, a few of them may be mentioned briefly.

In one experiment with the winter material a number of the filaments were placed for 2 minutes in distilled water saturated with CO_2. When centrifuged for 2 minutes at the same rate as that employed with the Protozoa, and then examined with the microscope, the chloroplasts in practically every cell were found to be aggregated in dense masses at the sides or ends of the cells, according to the position of the filament during centrifugalization. Controls in ordinary distilled water showed a much less complete separation. On the other hand, in filaments exposed for 10 minutes there was practically no movement of the chloroplasts at all, their spiral form being maintained almost as well as in cells which had been fixed for a few minutes in boiling water before centrifuging.

In another experiment filaments of *Spirogyra* were exposed to a saturated solution of carbon dioxide for 2, 4, 8 and 16 minutes,

respectively. The four lots, together with a control, were then centrifuged as before, with the following results: In the control some cells showed a separation of the chloroplasts, while others did not; in those exposed for 2 minutes practically all showed complete separation; in those exposed for 4 and for 8 minutes there was somewhat less separation (the amount being approximately the same as in the control), while with the 16-minute exposure there was very little separation and the filaments showed a certain apparent brittleness, being to some extent broken into pieces during their transfer to a watch glass for examination.

As to the reversibility of these effects on *Spirogyra,* it may be said that observations on this point are much more difficult to make than in the case of the motile Protozoa, and in the instances mentioned satisfactory determinations were not made. It has since been found with the more abundant material recently available that the filaments in which the exposure has been sufficient to produce decided liquefaction are not at all injured by the process, continuing to grow after the treatment in a normal manner. But whether or not recovery is possible after solidification can be determined only when *Spirogyra* which shows this behavior as clearly as did the first lot studied is again available in considerable quantities.

Discussion of Results.

It appears from the results obtained on four animal cells and on one plant cell that carbon dioxide can bring about changes in protoplasmic viscosity of two sorts. Clear evidence of liquefaction is obtained in *Paramœcium, Colpidium* and *Spirogyra.* Observations on this point are not available for *Arbacia* eggs, and in the case of *Amœba,* while the results are apparently in agreement with those already mentioned, they are based on mere appearances, and are therefore perhaps not so certain as the others. In every case where liquefaction was shown to occur this change could be shown to be reversible.

As to solidification, the clearest case is furnished by *Paramœcium,* where the process and its complete reversibility up to a certain point can easily be demonstrated. Conditions in *Colpidium* are almost as favorable, except that on account of the greater diffi-

culty in bringing about separation of the food vacuoles in the controls, the increasing viscosity can not be followed as far as in *Paramœcium*. In *Arbacia* eggs it is easy to produce solidification, which in the cases here described was in every case reversible, at least in the physical sense. On account of the complicating effects of artificial parthenogenesis, the physiological reversibility is more difficult to demonstrate, but in a number of instances it was shown in an entirely satisfactory manner. In one lot of *Spirogyra* solidification was obtained, but could not be produced in several other lots subsequently studied, and until favorable material is again available it will be impossible to make any definite statements about the reversibility of the process. Finally, in *Amœba*, all of the appearances, at least, of solidification can be produced, though in this animal it is impossible to test the matter by the method of centrifugalization.

Considering in its entirety the evidence which has been given, there seems to be no doubt that carbon dioxide is an efficient agent for changing the consistency of protoplasm reversibly in either direction, the exact effects produced depending on the concentration of the gas, the material experimented upon, the time of exposure and the presence or absence of other dissolved substances in the medium. Since CO_2 has these properties, and since it is so universally present in cells and is produced at different rates at different times, it seems not unlikely that it may prove to be an important factor in bringing about certain of the changes in viscosity that accompany various physiological activities of cells. In the case of dividing eggs the temptation is strong to attempt to correlate the striking variations in the rate of CO_2 production observed by Lyon ('04) with the equally striking changes in protoplasmic viscosity described by Heilbrunn ('20), Chambers ('17) and others. The chief difficulty in this case would appear to be that since carbon dioxide can have either a liquefying or a solidifying effect, the exact nature of the changes produced by it would be difficult to predict in advance.

In the case of amœboid movement also the hypothesis that carbon dioxide may be at least an important agent is an attractive one. As a matter of fact, L. Loeb ('20) and Hyman ('17) have both expressed the idea that some substance is produced as the result of

metabolism which causes the necessary changes in protoplasmic consistency. L. Loeb ('20) has further suggested that a change in hydrogen-ion concentration is also concerned. It will be noted that carbon dioxide satisfies both of these requirements. In addition, the evidence presented in the present paper shows that it is capable of producing exactly the sort of changes in protoplasmic consistency demanded by such a theory.

It would be easy to engage in further speculations about the probable rôle of CO_2 in other physiological processes, but such speculations would not be warranted by the present state of our knowledge. It is suggested, however, on account of its universal occurrence in cells, as well as on account of its interesting and in some respects unique physiological peculiarities, that carbon dioxide should at least receive serious consideration in connection with many of the as yet unsolved physiological problems which involve changes in protoplasmic consistency,

Summary.

1. A short exposure to carbon dioxide of various cells causes a decrease, and a longer exposure an increase in the viscosity of the protoplasm.

2. Both of these effects are reversible, though the second one tends to pass into an irreversible coagulation if the exposure is sufficiently long continued.

3. The local application of carbon dioxide to *Amœba* can influence in a striking way the formation of pseudopodia.

4. It is suggested that carbon dioxide may be an important factor in producing many of the natural changes in protoplasmic consistency which have hitherto been unexplained.

Literature Cited.

Bayliss, W. M.

'20 Colloidal Systems. IV. Reversible Gelation in Living Protoplasm. Proc. Roy. Soc., Vol. 91B, pp. 196–200.

Chambers, Robert, Jr.

'17 Microdissection Studies. II. The Cell Aster: a Reversible Gelation Phenomenon. Journ. Exp. Zoöl., Vol. 23, pp. 483–505.

Heilbronn, A.

'14 Zustand des Plasmas und Reizbarkeit. Jahrb. f. wiss. Bot., Bd. 54, pp. 357–390.

Heilbrunn, L. V.

'15 Studies in Artificial Parthenogenesis. II. Physical Changes in the Egg of *Arbacia*. BIOL. BULL., Vol. 29, pp. 149–203.

'20 An Experimental Study of Cell Division. I. The Physical Conditions which Determine the Appearance of the Spindle in Sea Urchin Eggs. Journ. Exp. Zoöl., Vol. 30, pp. 211–237.

Hyman, Libbie H.

'17 Metabolic Gradients in Amœba and their Relation to the Mechanism of Amœboid Movement. Journ. Exp. Zoöl., Vol. 24, pp. 55–99.

Jacobs, M. H.

'20*a* To what Extent are the Physiological Effects of Carbon Dioxide due to Hydrogen Ions? Amer. Journ. Physiol., Vol. 51, pp. 321–331.

'20*b* The Production of Intracellular Acidity by Neutral and Alkaline Solutions Containing Carbon Dioxide. *Ibid.*, Vol. 53, pp. 457–463.

Loeb, Leo.

'02 On the Blood Lymph Cells and Inflammation Processes in *Limulus*. Journ. Med. Research, Vol. 2, pp. 145–158.

'20 The Movements of the Amœbocytes and the Experimental Production of Amœbocyte (Cell-Fibrin) Tissue. Washington Univ. Studies, VIII, Science Series, No. 1, pp. 3–79.

'21 Amœboid Movement, Tissue Formation and Consistency of Protoplasm. Amer. Journ. Physiol., Vol. 56, pp. 140–167.

Lyon, E. P.

'04 Rhythms of CO_2 Production during Cleavage. Science, N.S., Vol. 19, pp. 350–353.

'07 Results of Centrifugalizing Eggs. Arch. f. Entwicklungsmechanik, Bd. 23, pp. 151–173.

Szücs, J.

'13 Ueber einige charakteristische Wirkungen des Aluminiumions auf das Protoplasma. Jahrb. f. wis. Bot., Bd. 52, pp. 269–332.

THE VITAL LIMIT OF EXSICCATION OF CERTAIN ANIMALS.

F. G. HALL,

ZOÖLOGICAL LABORATORY, UNIVERSITY OF WISCONSIN.

CONTENTS.

		Page
I. Introduction		31
II. Method of determining the vital limit		33
Apparatus		33
Plan of experiments		36
Water content determination		37
III. Experimental results		37
Annelida—	Earthworm	37
	Leech	39
Insecta—	Meal worm	40
Amphibia—	Salamander	41
	Frog	41
Reptilia—	Snake	43
	Turtle	43
	Lizards	44
Mammalia—	Mice	45
IV. General discussion and summary		48
V. Bibliography		50

INTRODUCTION.

Water is one of the most important constituents of living matter. The water content of animals varies from 50 to nearly 100 per cent. of the total body weight. All living matter appears to live in water. Not only is this true of the simplest forms of life, the Protozoa and the Protophyta, which are hardly more than naked protoplasm, but it is also true of the more complex organisms where lymph, blood, and sap are found as liquids; the immediate environment of the individual cell. Any change in the amount of water present may interfere with the activities of the cell. Yet it is a familiar fact to zoölogists that many animals will endure considerable exsiccation.

One hundred and fifty years of experimentation has shown that some animals become immobile when gradually dried out, and are apparently dead. This condition Preyer (1891) called "anabiosis." Leeuwenhoek was perhaps the first to observe this phenomenon in 1701. He found that Rotifera and Tardigrada

living in the sand of roof gutters could be completely dried out without losing vitality. Baker (1764) revived nematodes after they had been in a dried state for twenty-seven years. Doyère (1842) found that Tardigrada could endure higher temperatures, as well as extreme exsiccation, without injury and in many cases were able to live even in the absence of oxygen. Semper (1881) tells of experiments with *Apus* and *Cypris* eggs, which he kept in a chest of dried mud for six years. He was able to hatch out larvæ in summer or winter by adding water to the mud. Semper did not believe, however, that the eggs of the many animals that endure extreme exsiccation lose all their water, but that the integument of the egg does not permit the complete removal of water.

Professor W. S. Marshall has kept Trogoderma larvæ alive for more than five years in vials without food or water. Wodsedalek (1921) carried out similar experiments with the same species. The larvæ moulted but became smaller after each moult. They did not eat their moulted skins. In Nature's larger laboratory there are many cases of adaptation of animals to a dry environment. Protozoa and other microscopic forms encyst themselves when ponds dry out. Thus they resist in a motionless state the hot dry winds of summer, but revive again in spring when ponds are filled with water. Spores of bacteria are extremely resistant to exsiccation, living for days on dry glass. Desert lizards live in extremely dry places. In fact, a number of animals live in the driest places on earth.

While there are many experiments to show the effects of exsiccation most of them have been with simpler or smaller animals. Because of the microscopic size of these no quantitative results could be obtained. It would seem improbable that an animal can remain active after losing all its water, or that all the water could be removed from an organism without causing death. Thus it is important to study comparatively the amount of water that can be removed from animals without loss of vitality. Schmidt (1918) found that earthworms can revive and apparently become perfectly normal after a loss of 61.8 per cent. of the body weight, or nearly 73 per cent. of the weight of the water contained in the

body. He used the earthworm *Allolobophora foetida* and a desiccator containing calcium chloride. Durig (1901) found that the common European frog would lose 30 to 39 per cent. of the body weight if the drying was slow. Hill (1906) states that if a man loses ten per cent. of his weight in water, he usually dies. Shelford (1913) found in studying the reactions of animals to evaporation that smaller animals died from loss of water much more rapidly than larger. He also states that when the skins of amphibians became dry they did not recover when put into water.

This paper describes the exsiccation of certain animals. Experiments were carried on at the University of Wisconsin under the direction of Professor A. S. Pearse to whom the writer is greatly indebted for many suggestions and criticisms. Professor M. F. Guyer has also given numerous helpful suggestions. Thanks are also due to Dr. H. H. T. Jackson for the identification of the mammals used, and to Drs. J. A. E. Eyster and W. J. Meek for help in the construction of apparatus.

II. Method of Determination of the Vital Limit.

Apparatus.

The apparatus for these experiments consisted of an air-pump, exsiccating jars, filters, and an exsiccating chamber (Fig. 1).

The air-pump was an automatic electric air compressor[1] provided with an air-tank and a pressure gauge. The tank had a capacity of four gallons. A switch controlled the starting and stopping of the motor, whenever the pressure fell to 20 lbs. or rose to 25 lbs. A valve constructed on the air-tank allowed whatever flow of air was deemed necessary. The air-pump was always in the same room with the exsiccating chamber and where only fresh air was pumped and used.

The exsiccating jars (Fig. 1, *P, S*) consisted of four glass jars. The first (Fig. 1 *P*) was a 500 cc. Woulff bottle, full of crushed pumice stone, the pieces ranging from 0.5 mm. to 5 mm. in diameter, and about half full of sulfuric acid C.P. The other three jars were the ordinary washing bottles, filled with sulfuric acid C.P. The four jars were connected in series. Air passing

[1] Central Scientific Co. Cat. No. 218, listed as No. 1390.

from the compressed air-tank through a trap to prevent back flow of acid was rendered dry by its passage through the exsiccating jars.

Two filters were arranged. The first (Fig. 1, *Z*), a 100 cc. bottle containing about an equal weight of powdered and granulated zinc. The purpose of this filter was to prevent the passage of sulfuric acid. The second filter (Fig. 1, *G*), consisting of a 500 cc. bottle filled with glass wool, prevented the passage of any physical impurities. Tests of the air were made by allowing the air as it passed out of the exsiccating chamber to bubble through a methyl-orange solution for an hour. There were no indications of sulfuric acid in the air.

The manometer or flow-meter (Fig. 1, *M*) used for the determination of the rate of flow of air into the exsiccating chamber was constructed of glass tubing. A horizontal tube connecting the two ends of a U-tube and having a small aperture between the points of connection allowed the passage of air from the second filter to the exsiccating chamber. The U-tube was partly filled with olive oil, which was used because of its low evaporating coefficient. The passage of air through the horizontal tube increased the pressure on the column on one side thereby lowering the level. The difference of level of the liquid in the U-tube was a measure of the static pressure and was an indicator of the rate of flow of the air passing into the exsiccating chamber. The manometer was accurately calibrated from a gas meter used for such purposes by the department of physiology, University of Wisconsin. A scale was placed behind the U-tube and the rate of flow in cubic centimeters could be read at any time.

A large Whithall-Tatum museum jar (Fig. 1, *C*) was used for the exsiccating chamber. Four holes were drilled through the lid, one for the dry air inlet, one for the air outlet, two for the inlet and outlet tubes to the dew-point apparatus. The lid was fastened by a metal clamp and sealed by a rubber gasket. An outlet tube connected the exsiccating chamber with a small beaker containing sulfuric acid (Fig. 1, *O*). The end of the outlet tube was just far enough below the surface of the acid to indicate by bubbling if the chamber was tightly sealed, and also to prevent back flow of air containing water.

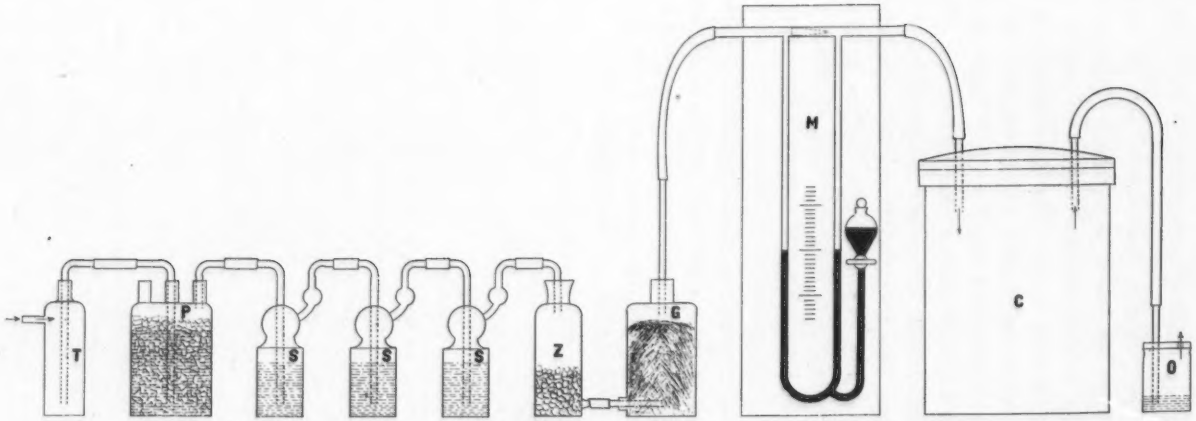


FIG. 1. Plan of apparatus for determining the vital limit of exsiccation of animals. *T*, trap; *P*, pumice stone and sulfuric acid exsiccating jar; *S*, sulfuric acid exsiccating jars; *Z*, zinc filter; *G*, glass wool filter; *M*, manometer; *C*, exsiccating chamber; *O*, outlet.

Plan of Experiments.

The animals used for these experiments, with the exception of the mice, were kept for several days without food; so that their digestive systems were evacuated. They were kept in cages and supplied constantly with water. The mice were kept in cages, and supplied with food and water. All the animals were weighed separately; the earthworms, leeches, and meal worms were weighed and then placed in small flat glass dishes which were covered with cheese cloth. From one to eight dishes were placed in the exsiccating chamber at a time; the salamanders, frogs, snakes, lizards, turtles, and mice were weighed in screen cages and transferred directly to the exsiccating chamber. A screen partition was used to separate the animals when more than one was exsiccated at a time.

The temperature, air-flow in cubic centimeters per minute, and the time were noted on a record card for each individual. The corn or oats fed the mice was weighed and the amount eaten noted each day. The relative humidity was found to vary considerably while animals were in the chamber. With an air-flow of 1,000 cubic centimeters per minute the relative humidity varied from 0 to 10 per cent.[1] The supply of sulfuric acid in the exsiccating jars was renewed whenever there was indication that it had been weakened much by the absorption of water.

After removal from the exsiccating chamber the animals were placed in as normal an environment as could be obtained. The invertebrates were placed on moist filter paper at normal room temperatures. Some individuals were placed in a refrigerator where recovery from exsiccation was more easily accomplished.

The limit of exsiccation was determined by the maximum percentage of water that could be removed from an animal without the loss of vitality. Water was removed from an animal by subjecting it to dry air in an exsiccating chamber (Fig. 1, *C*). The loss of weight was the means used for the determination of the

[1] By means of absorption tubes containing H_2SO_4, c. p. connected at the outlet of the exsiccating chamber (Fig. 1, *C*), not a trace of water was found to pass for a period of 25 hours, providing there was no animal in the exsiccating chamber.

percentage of water lost. Results of these experiments on the vital limit of exsiccation are summarized in Tables I.–XII.

Water Content Determination.

It was thought by the writer to be of interest to ascertain the amount of water normally present in the species that were used in the exsiccation experiments. The water content was determined by drying the animals in a drying oven and weighing at intervals until the weight became constant. The oven was kept at 99° C. The percentage of water in relation to the body weight for several species was as follows: meal worm (*Tenebrio molitor*), 49.8 per cent. (average of 60 individuals); leech (*Placobdella parasitica*), 76.4 per cent. (average of 10 individuals); house mouse (*Mus musculus*), 70.8 per cent. (average of 7 individuals); frog (*Rana pipiens*), 80.8 per cent. (average of 2 individuals). Schmidt (1918) found the water content of the earthworm, *Allolobophora fætida*, to be 84.1 per cent. (an average of 13 individuals).

III. Experimental Results.

Earthworm.

The species used in this experiment was the *Allolobophora chloroticus* (Savigny). The worms were collected near Lake Mendota during the summer of 1919. They were placed between moist filter paper in flat glass jars and kept for several days before using. They were rolled on dry filter paper in order to remove surface moisture and weighed in small flat dishes before being subjected to exsiccation. Several individuals were exsiccated at a time. Individual worms were removed at intervals. Directly following removal from the exsiccating chamber they were placed on moist filter paper for recovery.

When the worms were exsiccated they became contracted, assumed a dark brown color and lost all mobility. As exsiccation approached the vital limit the worms lost all elasticity of the body and a mucus-like covering was secreted. It was found that worms after exsiccation, if placed in a refrigerator at a temperature of 10° to 14° C., recovered after a greater loss of water than those that were kept at ordinary room temperature. Perhaps

lower temperature retarded bacterial decomposition. Worms Nos. 8–13, and 16–22, inclusive, did not show clitella. Many individuals showing clitella bled considerably. As the clitellum is abundantly supplied with blood vessels, blood effusion probably takes place more readily. Worms also appeared to be killed by infection in some cases.

The results of these experiments, with twenty-two individuals, show: (1) worms were exsiccated up to a loss of 69.6 per cent. of their body weight, or approximately 83 per cent. of the water contained in their bodies, was given up without loss of vitality; (2) lower temperatures were more favorable to the recovery of exsic-

TABLE I.

EARTHWORM, *Allolobophora chloroticus* (Savigny).

No.	Weight.		Condition of Exsiccation.			Results.			Condition of Animal after Exsiccation.
	Initial.	Lost.	Rate of Air-flow, $\frac{c.c.}{min.}$	Deg. C.	Time in Hours.	Survived.	Days Lived.	% Exsiccated.	
1	0.473	0.288	500	28	4′ 30″	no	—	61.1	Hard, dry, inactive
2	0.690	0.405	500	28	4′ 30″	no	—	42.7	Hard, dry, inactive
3	0.733	0.328	500	28	4′ 0″	no	—	44.7	Hard, dry, inactive
4	0.626	0.064	600	30	1′ 0″	yes	Indef.[1]	10.2	Active
5	0.570	0.150	600	30	2′ 0″	yes	Indef.	26.3	Active
6	0.223	0.144	600	30	3′ 0″	no	—	64.6	Inactive, dry, hard
7	0.199	0.139	600	30	3′ 0″	no	—	70.0	Inactive, dry, hard
8	1.833	0.870	400	28	2′ 30″	no	—	47.5	Inactive, dry
9	0.220	0.119	400	28	2′ 30″	no	—	54.1	Inactive, dry
10	0.240	0.149	300	28	1′ 30″	yes	2[2]	62.1	Alive, inactive (R)[2]
11	0.273	0.190	300	28	1′ 45″	yes	4	69.6	Inactive, dry (R)
12	0.171	0.091	300	28	1′ 45″	yes	Indef.	54.4	Inactive, dry (R)
13	3.014	0.780	500	27	1′ 45″	yes	1	25.9	Active (R)
14	0.590	0.115	500	27	2′ 0″	yes	Indef.	19.5	Active (R)
15	2.086	0.351	500	27	2′ 40″	no	—	16.8	Inactive, dry (R)
16	2.601	0.443	500	27	3′ 10″	yes	1	17.0	Active (R)
17	0.480	0.150	800	27	2′ 0″	yes	1	31.2	Active (R)
18	0.686	0.224	800	27	2′ 30″	yes	1	32.7	Active (R)
19	0.664	0.195	800	27	3′ 0″	yes	1	29.3	Active (R)
20	0.790	0.204	800	27	3′ 0″	yes	1	25.8	Active (R)
21	0.645	0.281	800	27	4′ 30″	yes	1	42.9	Inactive (R)
22	0.360	0.178	800	27	4′ 30″	yes	1	49.2	Inactive (R)

[1] By "Indef." is meant that the animals lived for forty days or more. The exact number of days was not recorded after the fortieth day.

[2] (R)—revived in refrigerator at 10° to 14° C.

[3] Death accidental.

cated worms; (3) worms not showing clitella dried more slowly and endured more exsiccation than those showing clitella.

Leech.

The species used for these experiments was *Placobdella parasitica* (Say) Moore. Specimens were collected from aquaria containing turtles and methods employed were the same as those used for earthworms with the exception that the leeches were kept under water until used. During exsiccation each leech coiled up into a ball-shaped mass containing air, for when they

TABLE II.

LEECH, *Placobdella parasitica* (Say) Moore.

No.	Weight.		Condition of Exsiccation.			Results.			Remarks.
	Initial, gms.	Lost, gms.	Time. Hours.	Deg. C.	Rate of Air-flow, $\frac{c.c.}{min.}$	Survived.	Days Lived.	% Exsiccated.	
1	0.620	0.323	6′ 15″	22	1,000	yes	6	52.1	R. (13° C.). Hard, dry, inactive.
2	0.865	0.524	6′ 15″	22	1,000	yes	8	60.6	Hard, dry, inactive.
3	0.592	0.403	6′ 0″	20	1,000	yes	5	68.1	Hard, dry, inactive.
4	1.470	0.520	8′ 0″	20	1,000	yes	Indef.	35.3	Dry, active.
5	0.733	0.403	5′ 30″	20	1,000	yes	6	55.0	Hard, dry, inactive.
6	0.542	0.350	8 0″	20	1,000	yes	Indef.	64.6	Hard, dry, inactive.
7	0.430	0.289	5′ 30″	20	1,000	yes	8	67.2	R. (13° C.). Hard, dry, inactive.
8	0.660	0.467	8′ 0″	20	1,000	no	—	70.7	Hard, dry, inactive.
9	0.484	0.341	7′ 30″	22	1,000	yes	17	70.4	R. (13° C.). Hard, dry, inactive.
10	0.566	0.242	5′ 30″	22	1,000	yes	Indef.	57.2	Hard, dry, inactive.
11	0.523	0.349	7′ 30″	22	1,000	no	—	66.7	Hard, dry, inactive.
12	1.175	0.540	9′ 45″	22	1,000	yes	Indef.	46.0	Dry, flexible.
13	0.554	0.370	6′ 15″	22	500	no	—	66.8	Hard, dry, inactive.
14	1.000	0.650	9′ 30″	22	500	no	—	65.0	Hard, dry, inactive.
15	0.476	0.326	9′ 30″	22	500	yes	4	68.7	R. (13° C.). Hard, dry, inactive.
16	0.331	0.256	9′ 30″	22	500	no	—	77.3	Hard, dry, inactive.
17	0.305	0.235	7′ 30″	25	1,000	no	—	77.0	R. (13° C.). Hard, dry, inactive.
18	0.320	0.250	7′ 30″	25	1,000	no	—	78.1	R. (13° C.). Hard, dry, inactive.
19	0.405	0.285	7′ 30″	25	1,000	no	—	70.4	R. (13° C.). Hard, dry, inactive.
20	0.425	0.313	7′ 30″	25	1,000	no	—	73.6	R. (13° C.). Hard, dry, inactive.

were dropped into water after exsiccation small air bubbles arose as the leeches uncoiled. After extreme exsiccation the leeches became hard; the skin dry; all mobility and elasticity were lost; and a mucus secretion enveloped the body. as was the case with the earthworms. During recovery the body, posterior to the twelfth segment, became swollen and was apparently inactive. The anterior part of the body remained active. The posterior part of the body became swollen to twice its natural size and softened. After a few days it broke open and the animal apparently bled to death. Leeches usually showed signs of recovery within an hour after removal from the exsiccation chamber to water.

The results of these experiments, with twenty individuals, show: (1) leeches were exsiccated to 70.4 per cent. of their body weight, or approximately 92 per cent. of the water contained in the body was given up, without loss of vitality; (2) lower temperatures were more favorable to the recovery of the exsiccated leeches.

Meal Worm.

The meal worm, the larva of *Tenebrio molitor* Linn., was used The methods employed were the same as with the earthworm. It was very difficult to tell when the vital limit of exsiccation was reached as the meal worms were inactive for the greater part of the time while in the exsiccating chamber. One meal worm lost a greater percentage of body weight than the average percentage of water contained in the body. The body of the meal worm contains much fat. It is probable that metabolic water was derived from this fat during exsiccation. The body of a meal worm always became considerably compressed during exsiccation.

The results of these experiments, with ten individuals, show: (1) meal worms were exsiccated to 52.6 per cent. of their body weight without loss of vitality; (2) the weight lost was greater than the average weight of water contained in the body; (3) the chitinous integument is probably a factor in offering resistance to evaporation. The meal worms endured exsiccation for a considerable length of time.

TABLE III.

MEAL WORM, *Tenebrio molitor* Linn.

No.	Weight.		Condition of Exsiccation.			Results.			Condition after Exsiccation.
	Initial.	Lost.	Rate of Air-flow, $\frac{c.c.}{min.}$	Temp. ° C.	Time, Hours.	Survived.	Days Lived.	% Exsiccated.	
1	0.223	0	500	22	4	yes	60+	0	Normal.
2	0.245	0	500	22	9	yes	60+	0	Normal.
3	0.190	0.002	500	22	49	yes	60+	1.0	Normal.
4	0.152	0.039	500	22	508	yes	60+	26.3	Inactive.
5	0.190	0.100	500	22	1,084	yes	2+	52.6	Inactive.
6	0.206	—	500	20	1,130	no	—	—	
7	0.195	0.185	500	20	70	yes	60+	5.1	Normal.
8	0.218	—	500	20	1,130	no	—	—	
9	0.190	0.007	500	20	70	yes	60+	3.7	Normal.
10	0.247	—	500	20	1,130	no	—	—	

Amphibia.

Two species of the Amphibia were used: the leopard frog, *Rana pipiens* (Schreber), and the salamander, *Ambystoma punctatum* (Baird). The frogs and salamanders were collected at various times during the year and were kept in a vivarium until used. Two or three specimens were exsiccated at a time, but individual records were kept. After extreme exsiccation neither frogs nor salamanders showed signs of life. There was not a reflex action to indicate that they were alive, though the temperature was 20° to 22°C. After they had been placed in water, following removal from the exsiccation chamber, during an hour they had always revived enough to swim. During one experiment weights were taken of a salamander to show how soon the weight lost could be regained. Within 24 hours the animal had regained 97.7 per cent. of the weight lost by the body during exsiccation.

The results of the experiments with salamanders, with nine individuals, show: (1) salamanders were exsiccated to 47 per cent. of their body weight without loss of vitality; (2) salamanders gave up water easily and regained water quickly when returned to their natural environment.

The results of the experiments with frogs, with six individuals, show: (1) frogs were exsiccated to 41.0 per cent. of their body weight, and that 50 per cent. of the water contained in their bodies was given up without loss of vitality.

TABLE IV.

SALAMANDER, *Ambystoma punctatum* Baird.

No.	Weight.		Condition of Exsiccation.			Results.			Condition and Behavior after Exsiccation.
	Initial, gms.	Lost, gms.	Deg. C.	Rate of Air-flow, c.c./min.	Time, Hours.	Survived.	Days Lived.	% Exsiccated.	
1	27.030	4.230	20	1,200	9′ 0″	yes	Indef.	15.6	Normally active.
2	37.500	4.300	18	1,000	13′ 30″	yes	Indef.	11.5	Normally active.
3	44.620	4.520	14–17	600	23′ 0″	yes	Indef.	10.1	Normally active.
4	38.600	4.800	14–17	600	23′ 0″	yes	Indef.	12.4	Normally active.
5	36.600	13.760	22	800	51′ 0″	yes	Indef.	37.6	Active, dried up appearance.
6	44.050	10.580	22	800	31′ 0″	yes	Indef.	24.0	Normally active.
7	23.370	11.170	8–20	500	188′ 0″	no	—	47.9	Dead, hard, dry.
8	35.550	15.750	8–20	500	144′ 0″	yes	Indef.	44.4	Inactive, dry, no signs of life.
9	38.450	18.070	20	600	116′ 0″	yes	Indef.	47.0	Inactive, dry, no signs of life.

TABLE V.

FROG, *Rana pipiens* Schreber.

No.	Weight.		Condition of Exsiccation.			Results.			Condition after Exsiccation.
	Initial, gms.	Lost, gms.	Rate of Air-flow, c.c./min.	Deg. C.	Time, Hrs.	Survived.	Days Lived	% Exsiccated.	
1	38.100	4.300	400	26	7	no	—	11.3	Inactive, dead.
2	37.000	3.900	400	26	7	yes	20+	10.5	Active.
3	22.800	9.340	1,000	22	33	yes	30+	41.0	Skin dry, apparently dead.
4	22.305	9.795	1,000	21	32	no	—	43.5	Inactive.
5	16.460	5.930	1,000	20	24	yes	30+	36.0	Apparently dead, skin dry.
6	18.230	8.270	1,000	21	31	no	—	45.6	Inactive.

Reptilia.

Five reptiles were used: the garter snake, *Thannophis radix* (Baird); the painted turtle, *Chrysemys marginata* Agassiz; the American chameleon, *Anolis carolinensis* Cuvier; the horned "toad," *Phrynosoma cornutum* Harlan; the desert lizard, *Sceloporus spinosus floridanus* Wiegmann. Only one snake was exsiccated and it died before removal from the exsiccation chamber.

The turtles were collected from the Madison lakes and were kept in a vivarium until used. As the shell of a turtle adds considerable weight to the body, the water content is not as high, and exsiccation cannot be carried as far. The turtles were revived from exsiccation by removal to an aquarium. Turtle No. 1 lost all activity after exsiccation. Its eyes were sunken, and the skin became very dry. The turtle appeared to be dead, but when cut open its heart was still beating.

The chameleons, horned "toads," and desert lizards were procured from a collector in Texas. The same method of exsiccation was employed as in the preceding experiments. No food was given. The chameleons were exsiccated at a comparatively rapid rate. Their skin became shrivelled, and eyes sunken. The exsiccation of the horned "toads" and desert lizards was very slow. The dry air apparently had no injurious effect upon them until a few days previous to death. At that time they gasped

TABLE VI.

TURTLE, *Chrysemys marginata* Agassiz.

No.	Weight.		Condition of Exsiccation.			Results.			Condition after Exsiccation.
	Initial.	Lost.	Rate of Air-flow, $\frac{c.c.}{min.}$	Deg. C.	Time. Hrs.	Survived.	Days Lived.	% Exsiccated.	
1	347.500	114.960	700	20	288	yes	1	33.1	Heart beating normally.
2	281.500	94.000	1,000	20	257	no	—	33.3	Inactive, dead.
3	334.000	94.800	1,000	20	304	yes	Indef.	28.4	Inactive, few signs of life.

continually and breathing seemed forced. As no food was given them, starvation was probably a factor in producing death. It is very remarkable that these animals can endure exsiccation for so long a time without food or water, in perfectly dry air. They lived for nearly four months, apparently with no discomfort. It is easy to understand how they can live in desert areas where many other animals would perish.

TABLE VII.

CHAMELEON, *Anolis carolinensis* Cuvier.

No.	Weight.		Condition of Exsiccation.			Results.			Condition after Exsiccation.
	Initial.	Lost.	Rate of Air-flow, $\frac{\text{c.c.}}{\text{min.}}$	Temp. ° C.	Time, Hours.	Survived.	Days Lived.	% Exsiccated.	
1	2.110	0.205	1,000	20	17	no	—	10.8	
2	2.360	0.880	1,000	20	184	yes	40+	28.8	Active.
3	1.910	0.885	1,000	20	186	yes	40+	46.3	Active.
4	1.315	—	1,000	20	48	no	—	—	
5	2.190	0.837	1,000	20	168	no	—	36.9	
6	1.220	0.350	1,000	20	195	yes	40+	28.8	Active.

TABLE VIII.

DESERT LIZARDS, *Phrynosoma cornutum* and *Sceloporus spinosus*.

Species.	No.	Weight.		Condition of Exsiccation.			% Exsiccated.	Survived.
		Initial.	Lost.	Rate of Air-flow, c.c./min.	Temp., ° C.	Time in Days.		
P. cornutum . .	1	31.510	10.640	800	21	119	33.8	no
" " . .	2	55.850	10.680	800	21	114	19.1	no
" " . .	3	41.250	—	800	21	22	—	no
S. spinosus . . .	1	38.300	11.340	800	21	86	29.6	no
" " . . .	2	9.400	4.500	800	21	62	47.8	no

The results of these experiments show: (1) Turtles were exsiccated to 33.1 per cent. of their body weight without loss of

[1] This specimen was killed after exsiccation for an examination of the anal bladder.

vitality; and chameleons were exsiccated to 46.3 per cent. of their body weight without loss of vitality. (2) Horned "toads" and desert lizards were exsiccated to 33.8 per cent. and 47.8 per cent. of their body weight, respectively, before death occurred. (3) Horned "toads" and desert lizards endured exsiccation and starvation for 119 and 86 days, respectively, before death occurred. (4) Reptiles are apparently better fitted to resist exsiccation than any other group of animals experimented upon.

Mammalia.

As representatives of the mammals, three rodents were used: the house mouse, *Mus musculus* (Linn.); the wood mouse, *Peromyscus leucopus noveboracensis* (Fischer); and the meadow mouse, *Microtus pennsylvanicus* (Ord.). The mice were caught in traps and kept in cages in a vivarium with food and water before them constantly. During exsiccation corn and oats were fed and the amount eaten determined by subsequent weighing. Mice remained active until the vital limit of exsiccation was apparently reached and then died quickly if not removed to a suitable environment. The mice became very nervous after subjection to the dry air for a few days but became drowsy as the vital limit was approached.

TABLE IX.

WOOD MOUSE, *Peromyscus leucopus noveboracensis* (Fischer).

No.	Weight.		Condition of Exsiccation.			Food.	Results.			Condition of Animal after Exsiccation.
	Initial.	Lost.	Rate of Air-flow, c.c. min.	Temp. ° C.	Time in Hours.	Gms. Oats; Corn.	Survived.	Days Lived.	% Exsiccated.	
1	14.120	4.900	1,000	20	144	10	no	—	34.7	Dead.
2	15.630	—	1,000	20	118	10	no	—	—	Dead.
3	11.790	3.440	1,000	20	75	3	yes	Indef.	29.2	Active.
4	12.230	3.760	1,000	21	77	20	yes	Indef.	30.7	Active.

The results of the experiments with mice, summarized in Tables IX.–XI., show: (1) The house mouse was exsiccated to

24.2 per cent. of its body weight, or about 34 per cent. of the water contained in its body was given up, without loss of vitality. (2) The wood mouse was exsiccated to 30.7 per cent. of its body

TABLE X.

MEADOW MOUSE, *Microtus pennsylvanicus* (Ord.).

No.	Weight.		Condition of Exsiccation.			Food.	Results.			Condition of Animal after Exsiccation.
	Initial.	Lost.	Rate of Air-flow, $\frac{c.c.}{min.}$	Temp. ° C.	Time in Hours.	Gms. Oats; Corn.	Survived.	Days cated.	% Exsic-Lived.	
1	32.110	9.930	1,000	20	48	2	yes	2	30.8	Active.
2	21.780	10.450	1,000	20	54	2	yes	1	48.0	Slightly active.
3	31.460	7.580	700	19	48	3	yes	Indef.	24.1	Active.
4	28.830	9.450	1,000	20	68	3	yes	Indef.	32.1	Active.

TABLE XI.

HOUSE MOUSE, *Mus musculus* Linn.

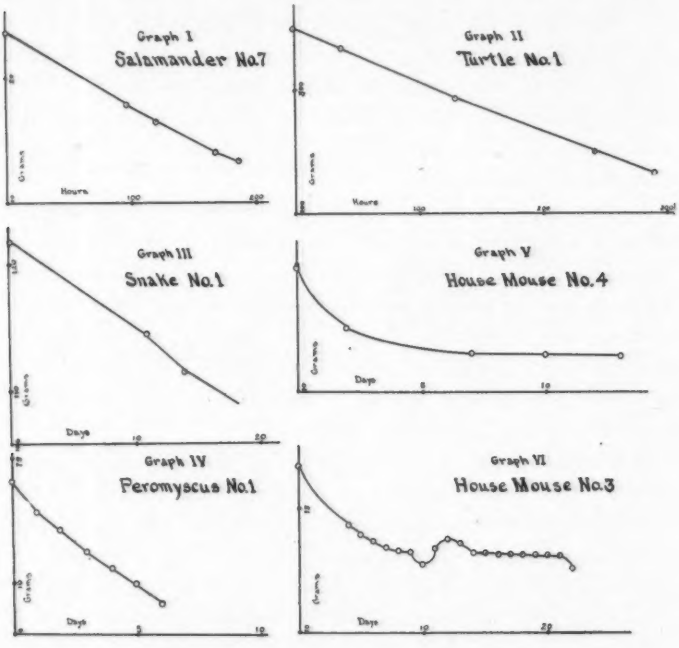
No.	Weight.		Condition of Exsiccation.			Food.	Results.			Behavior, Etc.
	Initial, gms.	Lost, gms.	Time, Hrs.	Deg. C.	Rate of Air-flow, $\frac{c.c.}{min.}$	Gms. Corn; Oats.	Survived.	Days Lived.	% Exsiccated.	
1	9.530	1.140	12	20	500	0	yes	Indef.	12	Normal.
2	13.820	3.200	65	20	600	0	1	1	23.3	
3	16.540	3.980	263	20	1,000	60	1	1	24.1	
4	14.860	3.460	308	20	1,000	25	1	1	23.3	
5	14.300	1.900	383	20	500	30	yes	Indef.	13.3	Inert, gasping for breath, at removal.
6	16.320	4.590	145	20	1,000	15	yes	—	28.1	Active.
6	16.320	5.735	191	20	1,000	15	no	—	35.1	Dead.
7	20.050	2.930	72	21	1,000	20	yes	Indef.	14.6	Active.
8	15.230	3.780	270	21	1,000	40	yes	Indef.	24.2	Active.

[1] These mice were dead when removed and the weight recorded is the last weight made before they died. As the exact time of death was not known, no weights of the dead mice were taken. The last weight was taken in each case from 12 to 24 hours before death.

weight without loss of vitality. (3) The meadow mouse was exsiccated to 32.1 per cent. of its body weight without loss of vitality.

Graphs.

The graphs shown in Fig. 2 represent the loss of water during exsiccation for intervals of time. It will be noted that in the graphs, with the exception of the house mouse, the water lost for intervals of time is constant. In the case of the house mouse, however (graphs V., VI.), the downward curve indicates a rapid



loss of water for the first few days, it then straightens and is nearly horizontal until death of the mouse occurs.[1] These facts indicate a resistance in the house mouse to evaporation—a resist-

[1] The irregularities in Graph VI. for the ninth to the twelfth days are due to the absence of food the ninth day and the excess eaten the following day.

ance that inhibits evaporation beyond a certain limit. Obviously, a mouse got some water from the corn eaten. The corn was tested and was found to contain less than eight per cent. water. Thus the amount of water obtained from the small amount of corn eaten would be too small to supply the daily loss. Probably metabolic water plays a part in replacing that evaporated.

Discussion.

The chief functions of water in animals are: (1) to dissolve nutrients, (2) to serve as a medium for their distribution, (3) to aid excretion by removing waste products from cells, and (4) to control the body temperature. Water is the most abundant constituent of the living organism and is essential to all life. The relative amount of water contained in animals depends upon the kind of organism, the period of growth, and the nature of food, environment and activities.

In these experiments an attempt has been made to determine the effect of a dry environment upon the amount of water present in organisms, and the effect of exsiccation upon the vital activities. There is need of more complete knowledge of the effects of exsiccation and though the present paper does not settle all problems relating to the loss of water by animals, it adds certain facts that may be of general interest. The different types of animals were subjected to comparable conditions.

It is apparent that the water content of different species varies greatly. The relative amount of water that may be lost and the relative length of time that animals can endure exsiccation also varies considerably. The important factors which limit such variations are: the kind of animal, the size of the animal, the integument, the general metabolism, and the elimination of body wastes.

The results of the writer's experiments apparently support the statements of Babcock (1912), that the need for water is much less for animals that excrete uric acid than those that excrete urea, since uric acid, being practically insoluble in the body fluids, is not as poisonous as urea, and is excreted in solid form with the loss of very little water. Weese (1912) has shown that the excretions of horned "toads" are largely uric acid. This is probably true for many insects.

The migration of the amphibians from water to land is regarded as a very important event in the evolution of animals. Closely associated with the adoption of a terrestial mode of existence by the amphibians is a change in the water content (Oswald, 1917). Before the frog takes to land, for instance, it consists of ninety-three per cent. water. When it becomes a land animal much of its water is lost. During its terrestial life a frog lives in moist places and when subjected to extreme dryness loses much of its water. In the writer's experiments as much as forty-one per cent. of the body weight, or fifty per cent. of the water contained in the body, was given up without loss of vitality. As water is given up so readily, areas where the evaporation rate is high would be generally unfavorable for frogs and for amphibians in general. Shelford (1913) has shown that amphibians, and other animals as well, react to evaporation gradients and concludes that the evaporating power of the air is probably the best index of the suitability of conditions for land animals.

Water does not evaporate from animals as it does from a surface of free water. Even those animals in which the integument permits little apparent evaporation, are able in some way to retard evaporation of water. To test this point a small stender dish containing 30 cc. of distilled water was subjected to the same conditions as a salamander during exsiccation. The area of surface exposed including skin, mouth, and lungs of a salamander weighing thirty-five grams, was estimated to be 121 square centimeters. The average amount of water lost from the dish having an area of 22.9 square centimeters was 4.520 grams per day. The average loss from salamanders was 0.770 grams per day. Of the animals used in the experiments described in this paper the salamander showed the most rapid rate of evaporation, yet the rate of evaporation from an exposed surface of free water was thirty times as great.

General Summary.

1. Animals in general are very susceptible to loss of water in atmospheres of low relative humidity.

2. Animals may lose a very large percentage of the water contained in their bodies without loss of vitality.

3. The amount of water that can be lost by animals without fatal results is in general greater in the less complex animals.

4. The length of time that animals can endure atmospheres of low relative humidity in general depends primarily upon the kind of integument and secondarily upon the proportion of the body surface to the body mass.

5. Metabolism of an organism is an important factor in its resistance to exsiccation.

TABLE XII.

COMPARATIVE RESULTS OF EXSICCATION EXPERIMENTS.

Species.	Table.	Per Cent. Exsiccated of		Time Required.
		Body Weight.	Water Content.	
Allolobophora chloroticus	I.	69.6	83	105 mins.
Placobdella parasitica	II.	70.3	92	450 mins.
Tenebrio molitor	III.	52.6	105	1,084 hours
Ambystoma punctatum	IV.	47.0	—	116 hours
Rana pipiens	V.	41.0	50	33 hours
Chrysemys marginata	VI.	33.1	—	288 hours
Anolis carolinensis	VII.	46.3	—	186 hours
Phrynosoma cornutum	VIII.	33.8	—	119 days
Sceloporus spinosus	VIII.	47.8	—	86 days
Peromyscus leucopus	IX.	30.7	—	77 hours
Microtus pennsylvanicus	X.	32.1	—	68 hours
Mus musculus	XI.	24.2	34	270 hours

BIBLIOGRAPHY.

Babcock, S. M.

'12 Metabolic Water: Its Production and Rôle in Vital Phenomena. University of Wisconsin, Agric. Exp. Sta. Research Bull. 22, Madison.

Bodine, J. H.

'21 Factors Influencing the Water Content and the Rate of Metabolism of Certain Orthoptera. Jour. Exp. Zoöl., Vol. 32.

Chenoweth, H. O.

'17 The Reactions of Certain Moist Forest Mammals to Air Conditions and Its Bearing on Problems of Mammalian Distribution. BIOL. BULL., Vol. 32.

Davenport, C. B.

'08 Experimental Morphology. New York.

Durig, A.

'01 Wassergehalt und Organfunction. Pfluger's Arch., 85.

Kudo, T.

'21 Studies on the Effects of Thirst. I. Effects of Thirst on the Weights of the Various Organs and Systems of Adult Albino Rats. Am. Jour. of Anatomy, Vol. 28.

Parker, G. H., and Parshley, H. M.

'11 The Reactions of Earthworms to Dry and Moist Surfaces. Jour. of Exp. Zoöl., Vol. 11.

Schmidt, P.

'18 Anabiosis of the Earthworm. Jour. of Exp. Zoöl., Vol. 27.

Shelford, V. E.

'13 The Reactions of Certain Animals to Gradients of Evaporating Power of Air. A Study in Experimental Ecology. BIOL. BULL., Vol. 25.

Schackell, L. E.

'09 An Improved Method of Desiccation with Some Applications to Biological Problems. Am. Jour. of Physiology, Vol. 24.

Weese, A. O.

'15 Urine in the Horned Toad. Science, Vol. 46.

Wodsedalek, J. E.

'12 Life History and Habits of *Trogoderma tarsale* (Melsh.). Annals of the Entomological Society of America, Vol. 5.